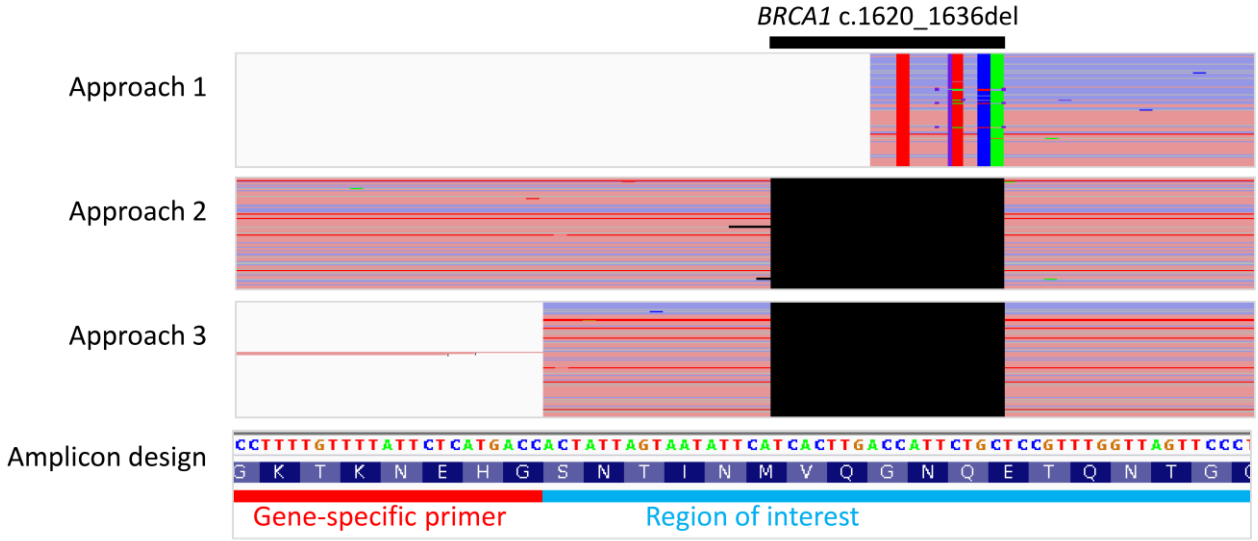
Supplementary Information for:

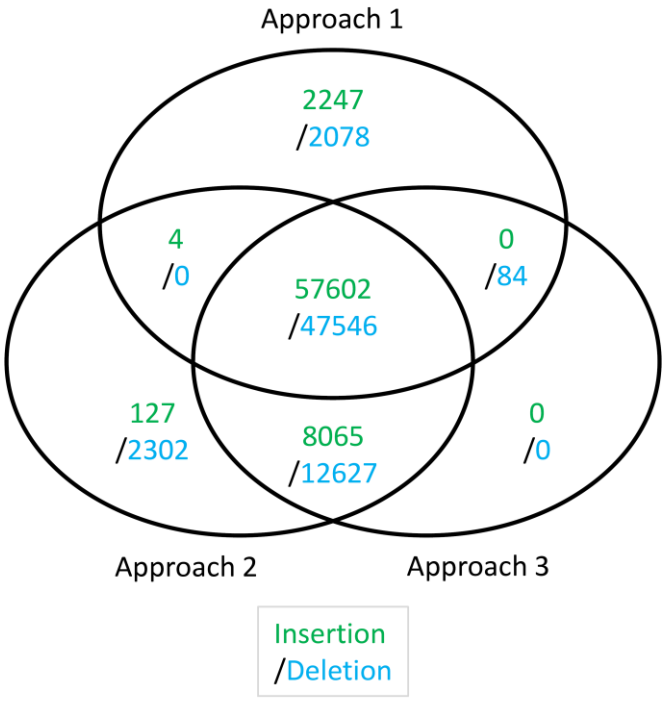
BAMClipper: removing primers from alignments to minimize false-negative mutations in amplicon next-generation sequencing

Chun Hang Au[1], Dona N. Ho[1], Ava Kwong[2,3,4], Tsun Leung Chan[1], Edmond S. K. Ma[1*]

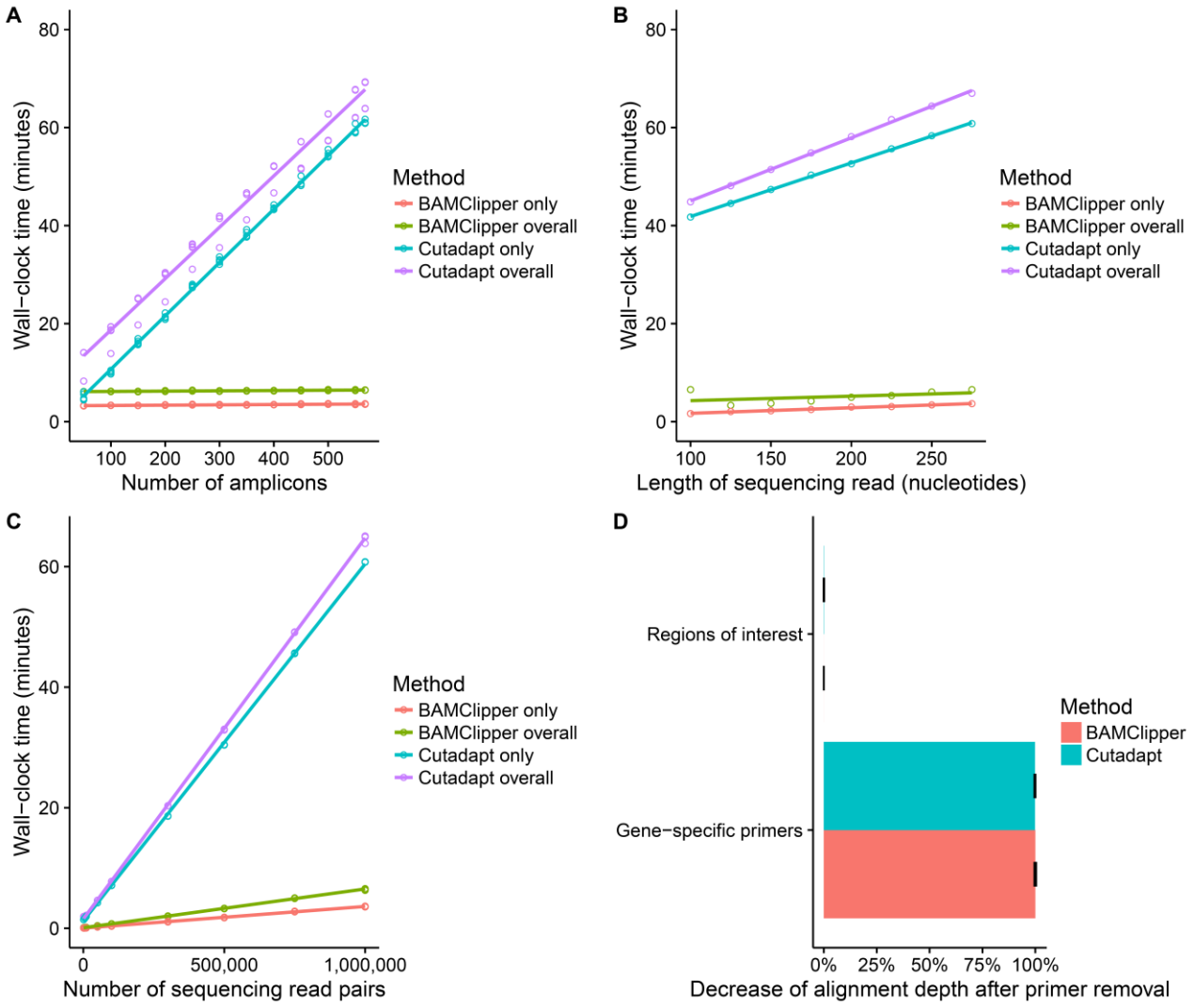
Supplementary Figure S1. A *BRCA1* deletion escaped from variant calling when primers were trimmed before mapping by BowTie 2. NGS read alignments of *BRCA1* c.1620_1636del allele from three primer handling approaches are shown in conjunction with the amplicon design and reference genome sequence. Individual forward and reverse sequencing reads after any soft-clipping were represented by red and purple horizontal lines, respectively. The expected deletion event (black box) was present in the alignments from approaches 2 and 3 only.



Supplementary Figure S2. Additional simulated indels detected by 3 approaches of primer handling.



Supplementary Figure S3. BAMClipper showed improved computing performance and maintained high effectiveness of primer removal. Computing time of 1 million read pairs for (A) increasing number of amplicons and (B) increasing length of sequencing read. (C) Computing time for increasing number of sequencing read pairs. A linear regression line is also shown for each method. (A-C) Computing time of BAMClipper or Cutadapt alone was shown as "BAMClipper only" and "Cutadapt only", respectively. Overall computing time from FASTQ to primer trimmed/clipped BAM files (including read alignment time) was shown as "BAMClipper overall" and "Cutadapt overall". (D) Mean decrease of alignment depth of region of interest or gene-specific primer sites of individual amplicons. Error bars represent 1 standard deviation.



Supplementary Table S1. Comparison of primer handling approaches in detecting known variants from the myeloid neoplasm gene panel.

Sample	Mutation^	Mutation type	Approach 1 (Cutadapt)	Approach 2	Approach 3 (BAMClipper)
1	*JAK2* c.1849G>T	SNV	Detected	Detected	Detected
2	*KIT* c.2447A>T	SNV	Detected	Detected	Detected
3	*MYD88* c.794T>C	SNV	Detected	Detected	Detected
4	*TP53* c.916C>T	SNV	Detected	Detected	Detected
5	*CALR* c.1154_1155insTTGTC	Insertion (5 nt)	Detected	Detected	Detected
6	*CALR* c.1124_1142del	Deletion (19 nt)	Detected	Detected	Detected
7	*CALR* c.1103_1136del	Deletion (34 nt)	Detected	Detected	Detected
8	*CALR* c.1099_1150del (hotspot type 1)	Deletion (52 nt)	Not detected due to soft-clipping	Detected	Detected

^Reference sequences: *CALR*: NM_004343.3, *JAK2*: NM_004972.3, *KIT*: NM_000222.2, *MYD88*: NM_002468.4, *TP53*: NM_000546.4

Supplementary Note. Source code of BAMClipper and Cutadapt pipelines used in benchmarking.

BAMClipper pipeline:

```
#!/bin/bash
# BAMClipper pipeline: FASTQ > BWA-MEM > BAMClipper
# ./bamclipper_pipeline_cray.sh samplename_R1.fastq.gz samplename_R2.fastq.gz
samplename trusight_myeloid.bedpe 16
#
R1=$1
R2=$2
NAME=$3
BEDPE=$4
NUMTHREAD=$5

STARTOVERALL=$(date +%s);
####

# bwa & index
START=$(date +%s);
bwa mem -R '@RG\tID:'$NAME'\tSM:'$NAME -M -t $NUMTHREAD -L 5 ucsc.hg19.fasta $R1 $R2
| samtools view -bS - | samtools sort -@ $NUMTHREAD -m 1536M > ${NAME}.bam && samtools
index ${NAME}.bam
END=$(date +%s);
echo "### bwa" $((END-START))

# bamclipper
START=$(date +%s);
./bamclipper.sh -b ${NAME}.bam -p $BEDPE -n $NUMTHREAD
END=$(date +%s);
echo "### bamclipper" $((END-START))

####
ENDOVERALL=$(date +%s);
echo "#### overall" $((ENDOVERALL-STARTOVERALL))
```

Cutadapt pipeline:

```
#!/bin/bash
# cutadapt pipeline: FASTQ > split > parallel cutadapt > merge > BWA-MEM
# ./cutadapt_pipeline_cray.sh samplename_R1.fastq.gz samplename_R2.fastq.gz
samplename cutadapt.R1-FR.opts cutadapt.R2-FR.opts 16
#
R1=$1
R2=$2
NAME=$3
CUTADAPTR1=$4
CUTADAPTR2=$5
NUMTHREAD=$6

STARTOVERALL=$(date +%s);
####

# zcat
START=$(date +%s);
zcat $R1 > ${NAME}_R1.fastq
zcat $R2 > ${NAME}_R2.fastq
END=$(date +%s);
echo "### zcat" $((END-START))

# split fastq (fastq-splitter is available from
http://kirill-kryukov.com/study/tools/fastq-splitter/)
START=$(date +%s);
perl fastq-splitter.pl --n-parts $NUMTHREAD ${NAME}_R1.fastq
perl fastq-splitter.pl --n-parts $NUMTHREAD ${NAME}_R2.fastq
END=$(date +%s);
echo "### split" $((END-START))

# parallel cutadapt
START=$(date +%s);
for i in $(seq -w 1 $NUMTHREAD); do
    CUTADAPT_OPTS_R1=`cat $CUTADAPTR1`
    CUTADAPT_OPTS_R2=`cat $CUTADAPTR2`
    echo "cutadapt $CUTADAPT_OPTS_R1 -m 0 -e 0.1 -n 2 ${NAME}_R1.part-$i.fastq -o
${NAME}_R1.part-$i.trimmed.fastq >/dev/null; cutadapt $CUTADAPT_OPTS_R2 -m 0 -e 0.1
```

```
-n 2 ${NAME}_R2.part-$i.fastq -o ${NAME}_R2.part-$i.trimmed.fastq >/dev/null" >>
$NAME.cutadapt.commands.list
done
parallel --joblog $NAME.cutadapt.parallel.log -j $NUMTHREAD <
$NAME.cutadapt.commands.list
END=$(date +%s);
echo "### cutadapt" $((END-START))

# merge
START=$(date +%s);
cat ${NAME}_R1.part-*.trimmed.fastq > ${NAME}_R1.trimmed.fastq
cat ${NAME}_R2.part-*.trimmed.fastq > ${NAME}_R2.trimmed.fastq
END=$(date +%s);
echo "### merge" $((END-START))

# bwa & index
START=$(date +%s);
bwa mem -R '@RG\tID:'$NAME'\tSM:'$NAME -M -t $NUMTHREAD -L 5 ucsc.hg19.fasta
${NAME}_R1.trimmed.fastq ${NAME}_R2.trimmed.fastq | samtools view -bS - | samtools
sort -@ $NUMTHREAD -m 1536M > ${NAME}.bam && samtools index ${NAME}.bam
END=$(date +%s);
echo "### bwa" $((END-START))


####
ENDOVERALL=$(date +%s);
echo "#### overall" $((ENDOVERALL-STARTOVERALL))
```